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Effect of low-dose irradiation on pregnant mouse hemopoiesis

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The effects of low-dose gamma radiation to hemopoietic progenitor cell compartments of the marrow and spleen of virgin female mice and pregnant mice were studied. Microplasma clot cultures were used to assess burst-forming unit-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E) activity, and double-layer agar cultures were established to evaluate granulocyte-macrophage colony-forming cell (GM-CFC) and macrophage colony-forming cell (M-CFC). The apparent shift in maternal erythropoiesis from the bone

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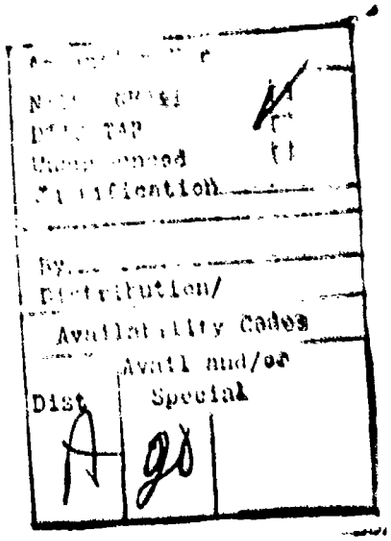
marrow to the enlarged spleen was reflected by an increase in the numbers of CFU-E and BFU-E per spleen and a concomitant decrease in CFU-E and BFU-E per femur. Whereas maternal GM-CFC values per femur increased 36%, maternal GM-CFC per spleen increased by 172% compared to virgin values. There was a greater decrease in M-CFC per spleen than per femur in the pregnant animal when values were compared to the virgin animal.

Total-body irradiation to the day-10.5 pregnant mouse caused a further suppression of day-14.5 medullary erythropoiesis (i.e., decreased CFU-E values) compared to the response of the virgin female mouse. An ability of the maternal spleen to support further compensatory erythropoiesis following increasing doses of radiation was demonstrated. Four days after 1.0 Gy exposure, maternal values for GM-CFC per femur or spleen decreased to nonirradiated virgin mice values. M-CFC per maternal femur decreased following 1.5 Gy, but M-CFC per spleen appeared to be unaffected with doses from 0.5 to 2.0 Gy.



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Effect of Low-dose Irradiation on Pregnant Mouse Haemopoiesis

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SUMMARY. The effects of low-dose gamma radiation to haemopoietic progenitor cell compartments of the marrow and spleen of virgin female mice and pregnant mice were studied. Microplasma clot cultures were used to assess burst-forming unit-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E) activity, and double-layer agar cultures were established to evaluate granulocyte-macrophage colony-forming cell (GM-CFC) and macrophage colony-forming cell (M-CFC). The apparent shift in maternal erythropoiesis from the bone marrow to the enlarged spleen was reflected by an increase in the numbers of CFU-E and BFU-E per spleen and a concomitant decrease in CFU-E and BFU-E per femur. Whereas maternal GM-CFC values per femur increased 36%, maternal GM-CFC per spleen increased by 172% compared to virgin values. There was a greater decrease in M-CFC per spleen than per femur in the pregnant animal when values were compared to the virgin animal.

Total-body irradiation to the day-10·5 pregnant mouse caused a further suppression of day-14·5 medullary erythropoiesis (i.e. decreased CFU-E values) compared to the response of the virgin female mouse. An ability of the maternal spleen to support further compensatory erythropoiesis following increasing doses of radiation was demonstrated. 4 d after 1·0 Gy exposure, maternal values for GM-CFC per femur or spleen decreased to nonirradiated virgin mice values. M-CFC per maternal femur decreased following 1·5 Gy, but M-CFC per spleen appeared to be unaffected with doses from 0·5 to 2·0 Gy.

Haemopoiesis in blood-forming tissues undergoes a temporary modification in the pregnant animal (Fowler & Nash, 1968; Fruhman, 1968; Rich & Kubanek, 1979). Alterations occur in the immunological responsiveness of the pregnant animal in order to support the developing fetus, which is considered an intrauterine allograft (Beer & Billingham, 1978; Nicklin & Billington, 1979; Stahn *et al*, 1978). The maternal transient anaemia probably caused by the nutrient and oxygen demands of the rapidly growing fetus has also been studied (Jepson, 1973). Effects of the existing environmental hazards that introduce additional stress to the

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physiological condition of the pregnant animal are gaining the attention of several investigators (Iversen *et al*, 1979; NCRP, 1977; Oppenheim *et al*, 1974). Experiments were conducted to elucidate further: (1) differences in erythropoietic and granulocytic-macrophage progenitor cell activity in the bone marrow and spleen of virgin and pregnant mice; (2) injury to maternal bone marrow and spleen size and haemopoiesis following total-body irradiation exposure during midpregnancy; and (3) compensatory erythropoiesis in the maternal spleen following different doses of ionizing radiation.

MATERIALS AND METHODS

Experimental Design

A comparative study was conducted with virgin female mice (♀), pregnant mice (♀), irradiated-virgin female mice, and irradiated-pregnant mice to assess the effect of low-dose irradiation on medullary and spleen cellularity, and the different haemopoietic progenitor cells. Experimental mice at 10·5 or 12·5 d of pregnancy and virgin females received a bilateral total-body irradiation (TBI) exposure of 0, 0·5, 1·0, 1·5, 2·0 or 3·0 Gy from the AFRRI cobalt-60 gamma radiation source at a rate of 0·4 Gy/min. All pregnant mice were killed on day 14·5 of pregnancy, and virgin mice were studied 4 d after irradiation for comparison. The data from four replicate experiments represent the injury manifestation and recovery potential at 2 or 4 d following TBI.

Mice. Virgin HA/ICR mice, 10–18 weeks old (Cumberland View Farms, Clinton, Tennessee), were randomly mated during a period of 24 h, designated as day 0 of gestation. All animals were maintained on a diet of Wayne Lab-Blox and acidified water (pH 2·5) *ad libitum* and housed in a facility with a 12 h light–dark cycle. All mice were screened for murine pneumonia complex and *Pseudomonas* spp. prior to each study.

Cell suspensions. Cell suspensions of spleen and flushed-out femurs from each mouse were prepared in a chilled medium that consisted of SAM* with 2% fetal bovine serum (heat-inactivated at 56°C for 30 min).

In Vitro Assays

Microplasma clot cultures were used to assess medullary and spleen erythroid progenitor cells as follows: aggregates of benzidine-stained-positive eight or more cells on day 3 of culture reflected the colony-forming units-erythroid (CFU-E), and several clusters of benzidine-stained-positive cells on day 9 of culture reflected the younger burst-forming units-erythroid (BFU-E). 1 ml plasma clot cultures consisted of 0·1 ml cell suspension (50 000 nucleated cells), 0·3 ml fetal bovine serum (heat-inactivated, Rehatuin F.S., Reheis Chemical Company, Illinois), 0·1 ml of beef embryo extract (McLeod *et al*, 1974), 0·1 ml of 10% bovine serum albumin (McLeod *et al*, 1974), 0·1 ml L-asparagine (final concentration 0·02 mg/ml) (McLeod

* SAM is Supplemented Alpha Modification of Eagle's Medium (Frank Monette, personal communication): 10·075 g Alpha Medium (Flow Labs); 10 ml nonessential amino acid solution (10 mM, 100× concentration, GIBCO); 10 ml sodium pyruvate solution (100 mM, 100× concentration, GIBCO); 10 ml L-glutamine (200 mM, 100× concentration, GIBCO); 20 ml penicillin (5000 units)-streptomycin sulphate solution (5000 mg) (Flow Labs); 1·87 g sodium bicarbonate powder; 950 ml tissue culture water (DIFCO). Final pH was adjusted to 7·5 with N_2OH. The 1000 ml of medium was prepared, millipore-filtered, aliquoted into 100 ml volumes, and stored in a refrigerator for no longer than 3 weeks.

et al, 1974), 0·1 ml 10^{-4} M 2-mercaptoethanol, and 0·1 ml erythropoietin (EPO) (anaemic sheep plasma, step III, Connaught Labs Inc., Swiftwater, Pennsylvania, lot no. 3023-3, 6·7 units per mg protein). All ingredients were maintained on ice and mixed with 0·1 ml bovine citrated plasma, maintained at 37°C, immediately prior to plating in microtitre wells (Cooke Engineering Co., Alexandria, Virginia). Cultures were then harvested, fixed, stained, and evaluated according to procedures outlined by McLeod *et al* (1979). Each ingredient was either reconstituted or diluted with SAM. For the control cultures, SAM was added instead of EPO. CFU-E cultures contained 0·125 units EPO per ml, and 3·0 units EPO per ml were used in BFU-E cultures. For each experimental group, six 0·1 ml microtitre well cultures were established and incubated at 37°C with humidified, 5% CO_2 in air for either 3 or 9 d. Mirotitre wells were sterilized by UV light for 3 h.

Double-layer soft agar culture technique outlined in detail by MacVittie (1979) was utilized to study the medullary and spleen granulocyte-macrophage colony-forming cell (GM-CFC) on day 10 of incubation and the macrophage-colony forming cell (M-CFC) appearing after 25 d of culture. Cultures were incubated at 37°C with humidified, 8% CO_2 in air. Pregnant mouse uteri extract (PMUE) was used as the source of colony-stimulating activity (CSA) for $3–5 \times 10^4$ bone marrow cells plated per ml or $0·5–1·0 \times 10^6$ spleen cells plated per ml in the agar cultures.

RESULTS

Tissue Cellularity

The effect of TBI on bone marrow cellularity and spleen cellularity is shown in Figs 1 and 2, respectively. Whereas 0·5 Gy diminished bone marrow cellularity of both groups of pregnant mice to about 41% of the nonirradiated pregnant mice, no effect was seen with irradiated virgin female mice. No significant difference was observed between marrow cellularity values of mice irradiated on day 10·5 or day 12·5 of pregnancy. Maternal spleen cellularity values were always more than 160% of nonirradiated or irradiated virgin female mice values. Commencing with 0·5 Gy damage to maternal spleen cellularity was reflected by day-12·5 irradiated group values. Recovery was indicated by the slightly higher day-10·5 irradiated pregnant mice group values, with the exception of 2·0 Gy group (Fig 2).

CFU-E and BFU-E

Mean values of erythroid committed stem cells of virgin mice were assessed 4 d after TBI. Virgin mice CFU-E per femur were greater compared to maternal mice ($P = 0·0025$) (Fig 3, Table I). 1 Gy and higher doses depressed the number of virgin female marrow CFU-E per femur and CFU-E per 10^5 cells ($P = 0·0005$). Day-10·5 pregnant mice irradiated with 1·5, 2·0 and 3·0 Gy resulted in a 58% decrease in absolute and relative medullary CFU-E values, compared to nontreated pregnant mice values. Pregnant mice spleens were outstandingly erythropoietically active compared to virgin mice spleens (indicated in Fig 4). Following a 20% increase in CFU-E per maternal spleen at 4 d after 0·5 Gy, values consistently decreased with increasing irradiation dose to levels below those in nonirradiated pregnant mice, but they were always higher than any virgin mice values.

Nonirradiated virgin female marrow BFU-E per 10^5 and per femur were higher than values

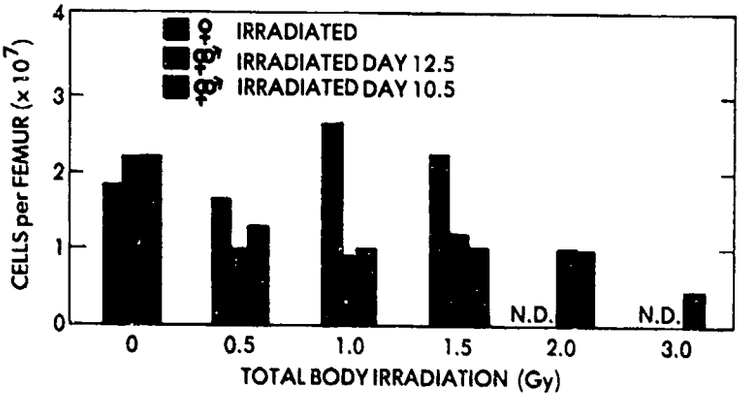

FIG. 1. Effect of total-body gamma irradiation on femoral marrow cellularity. Pregnant HA/ICR mice (♂) were irradiated on day 10·5 or 12·5 of gestation and killed on day 14·5. Virgin mice (♀) reflect 4 d post total-body irradiation. N.D. = not determined.

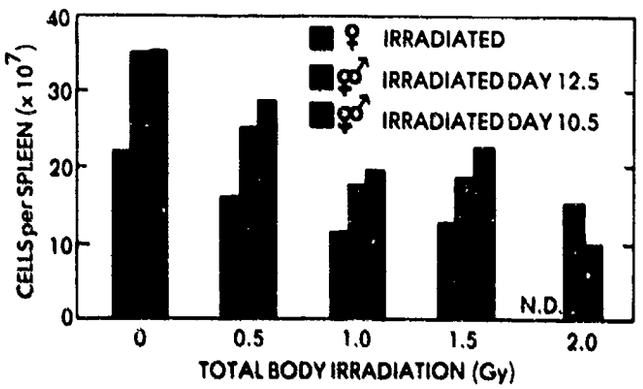

FIG. 2. Effect of total-body gamma irradiation on spleen cellularity. Pregnant HA/ICR mice (♂) were irradiated on day 10·5 or 12·5 of gestation and killed on day 14·5. Virgin mice (♀) reflect 4 d post total-body irradiation. N.D. = not determined.

for nonirradiated day-14·5 pregnant mice. Virgin and pregnant mice marrow BFU-E values were dramatically diminished with 0·5 Gy (values obtained 4 d following TBI) and were further decreased after 1·0 Gy (Fig 5). Pregnant mice BFU-E per 10^5 cells and per spleen were much higher than virgin mice values (Fig 5). 4 d following 0·5 Gy TBI, both virgin and pregnant mice had lower BFU-E values compared to that of nonirradiated mice. There was an increase in BFU-E (per 10^5 cells and per spleen) following 1·0 Gy in the maternal spleen, whereas virgin mice spleen BFU-E (per 10^5 cells and per organ) had reached their nadir level.

GM-CFC and M-CFC

Pregnant animals have 36% higher marrow GM-CFC per organ and 172% higher spleen GM-CFC per organ compared to values for virgin females (Fig 6, Table 1). With each increasing dose of irradiation administered on day 10·5, there was a decline in total marrow and spleen GM-CFC values of day-14·5 pregnant mice. 1 Gy appeared to reduce GM-CFC values

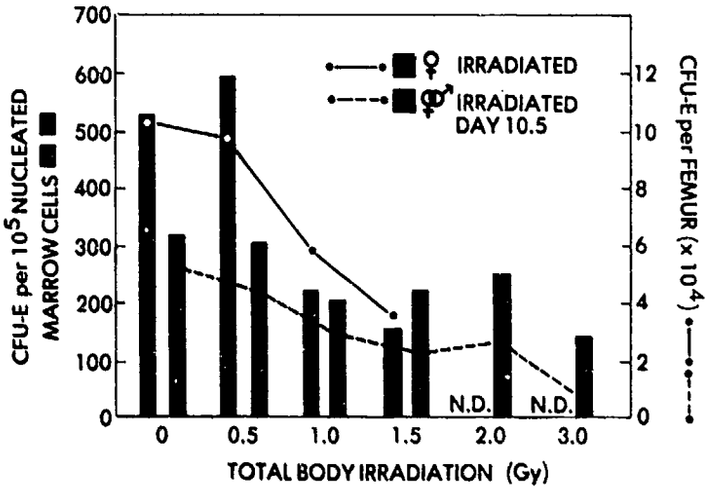

FIG 3. Effect of total-body gamma irradiation on femoral marrow CFU-E. Pregnant HA/ICR mice (♂) were irradiated on day 10·5 of gestation. Mean CFU-E values per 10^5 cells or per femur represent 4 d recovery of virgin (♀) and pregnant female mice. Erythropoietin, 0·125 u/ml, was added to plasma clot cultures containing 50 000 bone marrow cells per 0·1 ml. N.D. = not determined.

TABLE I. Comparison of organ cellularity and haemopoietic progenitor cells per organ in nonirradiated virgin HA/ICR female mouse (♀) and day-14·5 pregnant HA/ICR mouse (♂)•

	Bone marrow		*Spleen*	
	♀	♂	♀	♂
Organ cellularity (x 10^7)	1·8 ± 0·5	2·2 ± 0·2	21·8 ± 2·8	34·8 ± 4·2†
CFU-E (x 10^4)	10·4 ± 0·4	5·3 ± 1·3†	10·0 ± 8·2	21·2 ± 5·0
BFU-E (x 10^5)	24·3	16·0†	11·2	62·6†
GM-CFC (x 10^5)	3·3 ± 1·3	4·5 ± 1·7	1·1 ± 0·3	3·0 ± 0·8†
M-CFC (x 10^4)	3·9 ± 1·3	1·8 ± 1·2	3·3 ± 0·5	1·2 ± 0·2†

• Values represent the mean ± SEM.
† Statistically significant difference.

per organ to 50% of nonirradiated pregnant mice. This reduction in numbers of committed stem cells was maintained following 1·5 and 2·0 Gy TBI ($P = 0·05$). However, 3·0 Gy further reduced the medullary and spleen GM-CFC values. Concentration of GM-CFC (per 10^5 cells) remained relatively unchanged for maternal bone marrow following 0·5 and 1·0 Gy TBI. With a dose of 1·5 Gy there were 37% fewer GM-CFC per 10^5 marrow cells compared to the nonirradiated pregnant mice. Maternal spleen GM-CFC per 10^5 cells were more sensitive and were reduced to 27% of nonirradiated maternal values following 1·0 Gy and 76% of nonirradiated maternal values following 1·5 Gy.

The effect of pregnancy on M-CFC is different from that observed with GM-CFC or CFU-E. Marrow M-CFC per 10^5 and per femur values were similar for the nonirradiated

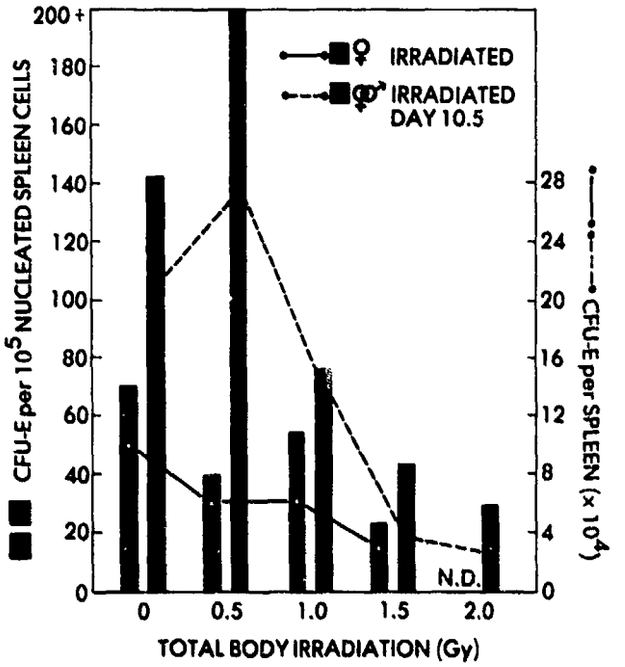

FIG 4. Effect of total-body gamma irradiation on spleen CFU-E. Pregnant HA/ICR mice (♀) were irradiated on day 10·5 of gestation. Mean CFU-E values per 10^5 cells or per spleen represent 4 d recovery of virgin (♀) and pregnant female mice. Erythropoietin, 0·125 u/ml, was added to plasma clot cultures containing 50 000 spleen cells per 0·1 ml. N.D. = not determined.

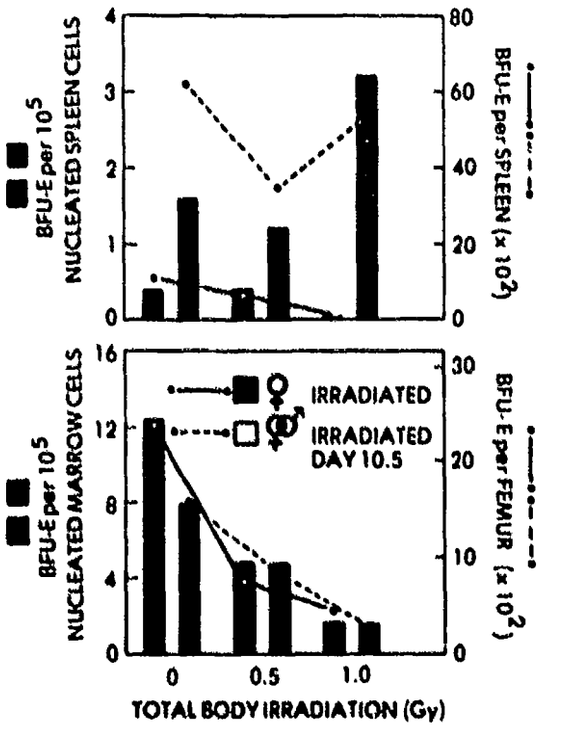

FIG 5. Effect of total-body gamma irradiation on femoral marrow BFU-E and spleen BFU-E. Pregnant HA/ICR mice (♀) were irradiated on day 10·5 of gestation. Mean BFU-E values per 10^5 cells or per femur or spleen represent 4 d recovery of virgin (♀) and pregnant female mice. Erythropoietin, 3·0 u/ml, was added to plasma clot cultures containing 50 000 bone marrow or spleen cells per 0·1 ml.

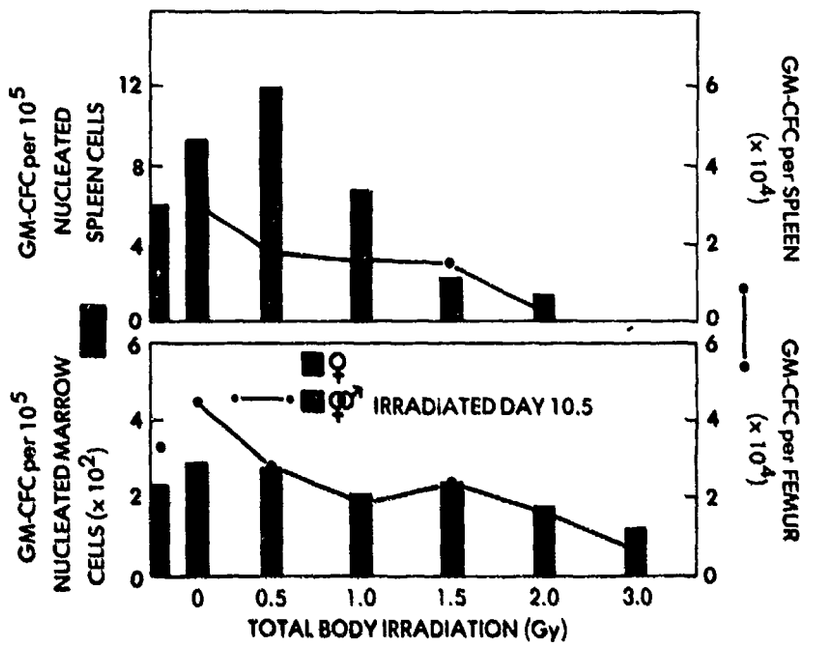

FIG 6. Effect of total-body gamma irradiation on femoral marrow and spleen GM-CFC. Pregnant HA/ICR mice (♀) were irradiated on day 10·5 of gestation and killed on day 14·5. Mean GM-CFC values per 10^5 cells or per femur or spleen are compared to nontreated virgin female mice (♀). Soft agar cultures contained PMUE and $3–5 \times 10^4$ bone marrow cells or $0·5–1·0 \times 10^6$ spleen cells per ml.

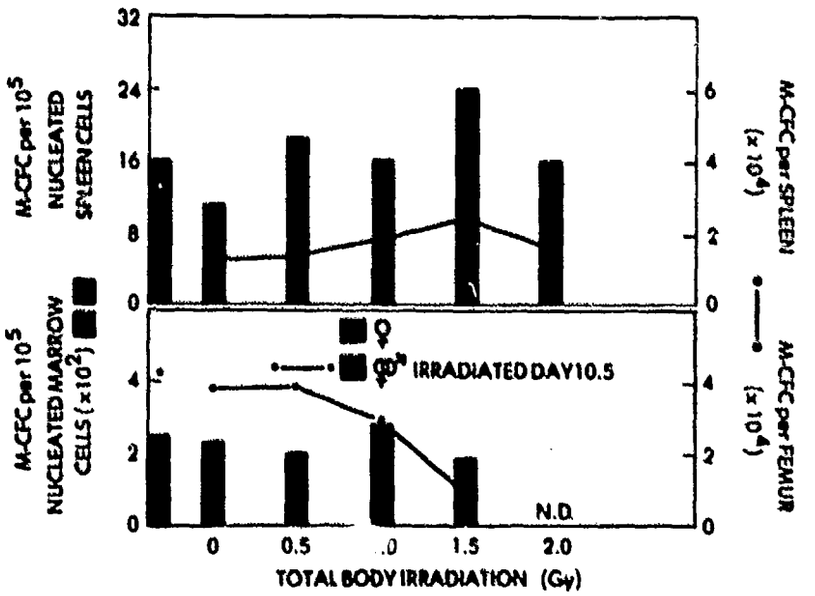

FIG 7. Effect of total-body gamma irradiation on femoral marrow and spleen M-CFC. Pregnant HA/ICR mice (♀) were irradiated on day 10·5 of gestation and killed on day 14·5. Mean M-CFC values per 10^5 cells or per femur or spleen are compared to nontreated virgin female mice (♀). Soft agar cultures contained PMUE, added on day 3 of culture, and $3–5 \times 10^4$ bone marrow cells or $0·5–1·0 \times 10^6$ spleen cells per ml. N.D. = not determined.

virgin and the nonirradiated pregnant mice. However, maternal M-CFC per spleen were significantly lower than the virgin female values ($P = 0{\cdot}0005$) (Fig 7, Table I). Total maternal marrow M-CFC values were reduced to 76% of nonirradiated maternal values with 1·5 Gy, whereas M-CFC per 10^5 cells remained relatively unchanged with increasing dose of irradiation. Unlike maternal marrow M-CFC, the spleen population of M-CFC was not affected by irradiation.

DISCUSSION

Our studies demonstrated a significant increase in spleen cellularity and a shift from marrow to spleen erythropoiesis in the day-14·5 pregnant animal. This was suggested by a decrease in marrow CFU-E and BFU-E, with a concomitant increase in CFU-E and BFU-E in the spleen. These observations are in agreement with other investigators (Mattsson *et al*, 1979; Rich & Kubanek, 1979) who reported splenic enlargement and haemopoietic activity during day 10 to day 16 of pregnancy. Fowler & Nash (1968) and Fruhman (1968) reported a gradually increasing murine bone marrow and spleen hyperplasia, along with enhanced erythropoiesis during the first 15 d. Ferrokinetic indices, peripheral blood RBCs, haematocrit percentages, and haemoglobin concentration values were used as the criteria to monitor erythropoiesis in the pregnant mouse. However, because the plasma clot culture system allows a more precise evaluation of the erythroid committed stem cell compartment in the blood cell-forming tissues, it was used in our studies.

Our studies also demonstrated that medullary GM-CFC were increased and M-CFC were not affected by the maternal physiological condition. In the maternal spleen there was an increase in GM-CFC and a decrease in M-CFC. The role of the macrophage in the immune reponse is well documented (Hibbs *et al*, 1978; Unanue & Calderon, 1975), and the modified immunocompetency of the pregnant animal (Beer & Billingham, 1978; Nicklin & Billington, 1979) to support the developing 'intrauterine allograft' may explain the observed decrease in the macrophage progenitor cell values relative to the virgin HA/ICR mouse.

The role of the spleen in supporting further compensatory haemopoiesis during pregnancy after the introduction of an additional stress (i.e. exposure to ionizing radiation) has not been investigated. Additional stress during early pregnancy of gamma radiation exposure caused a more pronounced effect on medullary haemopoiesis compared to the response of the virgin animal to similar doses of total-body irradiation. 4 d after irradiation of 0·5 Gy, the maternal femoral cellularity and CFU-E values were still significantly below control levels and below values for irradiated virgin mice. On the other hand, the maternal spleen appeared to be able to provide the increased compensatory haemopoiesis with greater CFU-E and BFU-E levels compared to the irradiated virgin female mouse.

The maternal spleen BFU-E and M-CFC response to increasing doses of irradiation was unlike the behaviour of the maternal marrow BFU-E and M-CFC. The bone marrow's rapid depletion of the haemopoietic progenitor cells after irradiation may be due to a 'sterilizing effect' of radiation, accompanied by an increase in differentiation and coupled with a migration of cells. The spleen, on the other hand, with its increase in BFU-Es and unchanged M-CFCs, may reflect the response of the spleen to an increased erythropoiesis demand and reduced macrophage activity during pregnancy.

Studies are presently being conducted with other laboratory animals in which the spleen is not haemopoietically active in order to gain more knowledge about the haemopoietic regulatory mechanisms involved in the low-dose-irradiated pregnant animal.

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REFERENCES

BEER, A.E. & BILLINGHAM, R.E. (1978) Immunoregulatory aspects of pregnancy. *Federation Proceedings*, **37**, 2374–2378.

FOLWER, J.H. & NASH, D.F. (1968) Erythropoiesis in the spleen and bone marrow of the pregnant mouse. *Developmental Biology*, **18**, 331–353.

FRUHMAN, G. (1968) Blood formation in the pregnant mouse. *Blood*, **31**, 242–248.

HIBBS, J.B., JR, CHAPMAN, H.A., JR & WEINBERG, J.B. (1978) The macrophage as an antineoplastic surveillance cell: Biological perspectives. *Journal of Reticuloendothelial Society*, **24**, 549–570.

IVERSEN, T., TALLE, K. & LANGMARK, F. (1979) Effect of irradiation on the feto-placental tissues. *Acta Radiologica et Oncologica*, **18**, 129–135.

JEPSON, J.H. (1973) Haematological disorders in pregnancy. In: *Clinics in Haematology*, Vol. 2. Saunders, Philadelphia.

MACVITTIE, T.J. (1979) Alterations induced in macrophages and granulocyte-macrophage colony-forming cells by a single injection of mice with *Corynbacterium parvum*. *Journal of Reticuloendothelial Society*, **26**, 479–490.

MATTSSON, R., NILSSON, B. & LINDAHL-KIESSLING, K. (1979) An investigation of splenic enlargement in pregnant mice. *Developmental Comparative Immunology*, **3**, 683–695.

McLEOD, D.L., SHREEVE, M.M. & AXELRAD, A.A. (1974) Improved plasma clot culture system for production of erythrocytic colonies *in vitro*: quantitative assay method for CFU-E. *Blood* **44**, 517–534.

McLEOD, D.L., SHREEVE, M.M. & AXELRAD, A.A. (1979) Culture systems *in vitro* for the assay of erythrocytic and megakaryocytic progenitors. *In Vitro Aspects of Erythropoiesis* (ed. by M.J. Murphy, Jr), pp. 31–36. Springer, New York.

NCRP, NATIONAL COUNCIL ON RADIATION PROTECTION AND MEASUREMENTS (1977) Medical radiation exposure of pregnancy and potentially pregnant women. NCRP Report No. 54, National Council on Radiation Protection and Measurements, Washington.

NICKLIN, S. & BILLINGTON, W.D. (1979) Macrophage activity in mouse pregnancy. *Journal of Reproductive Immunology*, **1**, 117–126.

OPPENHEIM, B.E., GRIEM, M.L. & MEIER, P. (1974) Effects of low-dose prenatal irradiation in humans: Analysis of Chicago lying-in data and comparison with other studies. *Radiation Research*, **57**, 508–544.

RICH, I.N. & KUBANEK, B. (1979) The ontogeny of erythropoiesis in the mouse detected by the erythroid colony-forming technique. I. Hepatic and maternal erythropoiesis. *Journal of Embryology and Experimental Morphology*, **50**, 57–74.

STAHN, R., FABRICIUS, H.A. & HARTLEITNER, W. (1978) Suppression of human T-cell colony formation during pregnancy. *Nature*, **276**, 831–832.

UNANUE, E.M. & CALDERON, J. (1975) Evaluation of the role of macrophages in immune induction. *Federation Proceedings*, **34**, 1737–1742.